FEE TRANSMITTAL FOR FY 2006

TOTAL AMOUNT OF PAYMENT ($) 1520

Complete if Known	
Application Number	09/715,902
Filing Date	11/17/2000
First Named Inventor	Donnelly et al.
Examiner Name	Wehbé, Anne Marie Sabrina
Group Art Unit	1632
Attorney Docket No.	PP01627.003

METHOD OF PAYMENT

1. [X] The Commissioner is hereby authorized to charge any fee due and to credit any overpayment to:

Deposit Account Number: 50-1047

Deposit Account Name: Mayer & Williams

[X] Charge Any Additional Fee required under 37 CFR 1.16 and 1.17

[ ] Applicant claims small entity status. See 37 CFR 1.27

2. [ ] Payment Enclosed: PTO-2038 enclosed

[ ] Check [ ] Credit Card [ ] Money Order [ ] Other

FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)		Fee Paid
1011	300	2011	150	Utility filing fee	
1012	200	2012	100	Design filing fee	
1013	200	2013	100	Plant filing fee	
1014	300	2014	150	Reissue filing fee	
1005	200	2005	100	Provisional filing fee	
1111	500	2111	250	Utility Search Fee	
1112	100	2112	50	Design Search Fee	
1113	300	2113	150	Plant Search Fee	
1114	500	2114	250	Reissue Search Fee	
1311	200	2311	100	Utility Examination Fee	
1312	130	2312	65	Design Examination Fee	
1313	160	2313	80	Plant Examination Fee	
1314	600	2314	300	Reissue Examination Fee	
1081	250	2081	125	Size Fee – ea addl 50 sheets over 100 sheets	

SUBTOTAL (1) ($)

2. EXTRA CLAIM FEES

		Previously Paid**		Extra Claims		Fee from below	Fee Paid
Total Claims	23	- 27	=	0	X	50	=
Independent Claims	1	- 4	=	0	X	200	=
Multiple Dependent						360	=

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	50	2202	25	Claims in excess of 20
1201	200	2201	100	Independent claims in excess of 3
1203	360	2203	180	Multiple dependent claim
1204	200	2204	100	Reissue independent claims in excess of 3
1205	50	2205	25	Reissue claims in excess of 20

SUBTOTAL (2) ($)

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	
1051	130	2051	65	Surcharge – late filing fee or oath	
1052	50	2052	25	Surcharge – late Provisional filing	
1053	130	1053	130	Non-English specification	
147	2520	147	2520	For filing a request for ex parte Reexam	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1840*	1805	1840*	Requesting publication of SIR after Examiner action	
1251	120	2251	60	Extension for reply within first month	
1252	450	2252	225	Extension for reply within second month	
1253	1020	2253	510	Extension for reply within third month	1020
1254	1590	2254	795	Extension for reply within fourth month	
1255	2160	2255	1080	Extension for reply within fifth month	
1401	330	2401	165	Notice of Appeal	
1402	330	2402	165	Filing a brief in support of an appeal	500
1403	290	2403	145	Request for oral hearing	
1451	1510	1451	1510	Petition to institute a public use proceeding	
1452	1330	2452	665	Petition to revive - unavoidable	
1453	1330	2453	665	Petition to revive - unintentional	
1501	1330	2501	665	Utility issue fee (or reissue)	
1502	480	2502	240	Design issue fee	
1503	640	2503	320	Plant issue fee	
1814	130	2814	65	Statutory Disclaimer	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of IDS	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	770	2809	385	Filing a submission after final rejection (37 CFR § 1.129(a))	
1810	770	2810	385	For each additional invention to be examined (37 CFR § 1.129(b))	
1801	790	2801	395	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify)

SUBTOTAL (3) ($) 1520

SUBMITTED BY

Complete (if applicable)

Name (Print/Type)	David B. Bonham	Registration No.	34,297	Telephone	703-433-0510
Signature				Date	9/5/06

Applicant:	Donnelly et al.
Serial No.:	09/715,902
Filed:	11/17/2000
Title:	MICROPARTICLE-BASED TRANSFECTION AND ACTIVATION OF DENDRITIC CELLS
Art Unit:	1632
Examiner:	Wehbé, Anne Marie Sabrina
Docket No.:	1627.003
Conf. No.:	5612

Mail Stop Appeal Brief-Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

PETITION UNDER 37 CFR 1.136(a) AND APPEAL BRIEF UNDER 37 C.F.R. §41.37

Applicants hereby petition the Commissioner to grant a three (3) month extension of time, up to and including Tuesday, September 5, 2006, in which to file an Appeal Brief following the Notice of Appeal filed on April 3, 2006 and received in the Patent Office on April 5, 2006.

This Appeal Brief is being submitted in response to the Examiner's final rejection of November 2, 2005. Appellant respectfully requests that the Board of Patent Appeals and Interferences reverse the Examiner's rejection of the claimed subject matter.

09/12/2006 AWONDAF1 00000076 501047 09715902

01 FC:1402 500.00 DA
02 FC:1253 1020.00 DA

I. REAL PARTY IN INTEREST

Novartis Vaccines and Diagnostics, Inc. (formerly Chiron Corporation) is the assignee of the present invention and the real party in interest.

II. RELATED APPEALS AND INTERFERENCES

No prior or pending appeals, interferences or judicial proceedings are known to appellant, the appellant's legal representative, or assignee which may be related to, directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

IIII. STATUS OF CLAIMS

Claims 1-16, 18-23, 29-31, 33-44, 46, 50, and 52-54 are pending and are provided in the attached Appendix. Claims 17, 24-28, 32, 45, 47-49, 51 have been cancelled.

Claims 1-16, 18-23, 29-31, 33-44, 46, 50, and 52-54 are rejected. Appellant hereby appeals the final decision of the Examiner rejecting claims 1-16, 18-23, 29-31, 33-44, 46, 50, and 52-54.

IV. STATUS OF AMENDMENTS

No amendments have been made subsequent to the Final Office Action mailed on November 2, 2005, rejecting claims 1-16, 18-23, 29-31, 33-44, 46, 50, and 52-54.

V. SUMMARY OF CLAIMED SUBJECT MATTER

The invention is defined in claims 1 and 54, the only independent claims, which read as follows:

1. (Previously presented) A method of transfecting dendritic cells comprising:

providing dendritic cells;

providing a transfection agent comprising polynucleotide adsorbed on surfaces of microparticles, said transfection agent being formed by a process that comprises: (a) providing microparticles comprising a biodegradable polymer and a cationic detergent, and (b) exposing said microparticles to said polynucleotide, said polynucleotide encoding an antigen associated with a virus, a bacterium, a parasite, a fungus or a tumor; and

incubating the dendritic cells and the transfection agent *ex vivo* for a time sufficient to transfect the dendritic cells with the polynucleotide, thereby leading to the expression of said antigen.

54. (Previously presented) A method of transfecting dendritic cells comprising: incubating dendritic cells and a transfection agent that comprises a polynucleotide, which encodes an antigen associated with a virus, a bacterium, a parasite, a fungus or a tumor, adsorbed on surfaces of microparticles, said incubating being performed *ex vivo,* for a time sufficient to transfect the dendritic cells with the polynucleotide, thereby leading to the expression of said antigen.

Thus, the claimed present invention provides an effective method for the transfection of dendritic cells by non-viral methods. Present specification, page 6, lines 13-19. The present invention provides this benefit by incubating dendritic cells and a specified transfection agent. *Id.* The transfection agent comprises polynucleotide and microparticles, with the microparticles being comprised of a biodegradable polymer. *Id.* The dendritic cells and transfection agent are incubated *ex vivo* for a time sufficient to transfect the dendritic cells with the polynucleotide. *Id.*

One advantage of the present invention is that polynucleotides can be efficiently internalized by dendritic cells. Present specification, page 7, lines 11-12.

Another advantage of the present invention is that gene expression can be effected within dendritic cells. Present specification, page 7, lines 13-14.

Yet another advantage of the present invention is that antigen can be processed and presented in connection with MHC molecules on the surface of dendritic cells. Present specification, page 7, lines 15-16.

Another advantage of the present invention is that polynucleotides can be rapidly internalized and expressed, with antigen presentation. Present specification, page 7, lines 17-18.

Still another advantage of the present invention is that the methods of the invention can be used, for example, in genetic immunotherapy or vaccination with relative safely. Present specification, page 7, lines 19 *et seq.*

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The following grounds of rejection are presented for review:

Claims 1-16, 18-23, 29-31, 33-44, 46, 50, and 52-54 stand finally rejected under 35 U.S.C. 103(a) as obvious over WO 97/24447 to Song et al. (Song) in view of U.S. Patent No. 5,783,567 to Hedley et al. (Hedley) and E. Fattal et al., *Journal of Controlled Release*, 53 (1998) 137-143 (Fattal) .

VII. ARGUMENT

Rejection of Claim 54

Claim 54 is rejected under 35 U.S.C. 103(a) as obvious over Song in view of Hedley and Fattal. This rejection is clearly erroneous for the reasons to follow.

Claim 54 is directed to a method of transfecting dendritic cells. The method comprises incubating dendritic cells and a transfection agent that comprises a polynucleotide (which encodes an antigen associated with a virus, a bacterium, a parasite, a fungus or a tumor) adsorbed on surfaces of microparticles. The incubation is performed *ex vivo* for a time sufficient to transfect the dendritic cells with the polynucleotide, thereby leading to the expression of said antigen.

"To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure." MPEP 2142, citing *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

Song describes compositions and methods useful for stimulating an immune response against one or more disease associated antigens by genetically modifying dendritic cells *in vivo* or *ex vivo*. See Song Abstract. Gene delivery vehicles are described, which are targeted to dendritic cells, whether *in vivo* or *in vitro*, and which comprise a dendritic cell targeting element and an expression vector which directs expression of at least one disease associated antigen. *Id.*

at page 2, lines 16-19. The dendritic cell targeting element can be any molecule which targets the gene delivery vehicle to a dendritic cell, for example, a high affinity binding pair, an antibody reactive against a dendritic cell surface marker, an antigen binding domain derived from an antibody reactive against a dendritic cell surface marker, or a hybrid envelope protein. *Id.* at page 3, lines 16-31.

In some embodiments, the expression vector is carried by a recombinant virus, including DNA and RNA viruses, preferably a recombinant virus derived from either a negative strand RNA virus or a positive strand RNA virus. *Id.* at page 2, lines 19-22. Various negative and positive strand viruses are set forth, for example, at page 2, line 22 to page 3, line 2. In other embodiments, the gene delivery vehicle is non-viral gene delivery vehicle. *Id.* at page 3, lines 5-7. In some of these embodiments, the expression vector is complexed with one or more polynucleotide condensing agents, including polycations. *Id.* at page 3, lines 8-10. In some of these embodiment, the expression vector is associated with lipids, preferably encapsulated in liposomes. *Id.* at page 3, lines 13-15. In other embodiments, the expression vector is complexed only with the dendritic cell targeting element. *Id.* at page 3, lines 12-13.

Thus, Song teaches that the gene delivery vehicle may be viral or non-viral (i.e., that any gene delivery vehicle will suffice), and that dendritic cells may be genetically modified *in vivo* or *ex vivo* (i.e., that any mode of modification will suffice). Song, however, clearly expresses a preference for *in vivo* (direct injection) delivery of recombinant retroviruses carrying an expression vector. *Id.* at page 27, lines 25-27. Furthermore, the non-viral vehicles taught by Song (i.e., polynucleotides associated with condensing agents or encapsulated in liposomes) are unrelated to the delivery vehicle of claim 54 (i.e., polynucleotides adsorbed on surfaces of microparticles), other than in the sense that they are "non-viral" techniques.

In this regard, it is a well settled tenant of patent law that "[t]he references must be considered *as a whole* and must suggest the desirability and thus the obviousness of making the combination. MPEP 2141, citing *Hodosh v. Block Drug Co., Inc.*, 786 F.2d 1136, 1143 n.5, 229 USPQ 182, 187 n.5 (Fed. Cir. 1986). (Emphasis added.)

Recognizing that Song is deficient, the Examiner argues that Hedley supplements Song through its teachings regarding the use of microspheres comprising biodegradable polymers and it's use of DNA plasmids to introduce and express antigens encoded by the plasmids in antigen presenting cells such as macrophages and dendritic cells, both *in vitro* and *in vivo*, for the

purpose of stimulating antigen specific immune responses. See the Office Action mailed September 22, 2004, page 5. It is further argued that Hedley provides motivation for introducing plasmid DNA encoding an antigen to antigen presenting cells such as macrophages and dendritic cells using biodegradable microspheres by teaching that DNA combined with biodegradable microparticles is efficiently phagocytosed by antigen presenting cells and is an effective means for introducing nucleic acids into these cells. *Id.* at page 6. The Examiner further argues that Hedley recognizes that dendritic cells are a "legitimate target" for microparticle transfection when stating that the point of introduction of plasmid/microparticles to skin is the transfection of dendritic cells. *Id.*

As noted above, the teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. The Examiner's view of Hedley, however, has clearly been colored by hindsight that has been gleaned from appellant's disclosure.

In particular, Hedley is said to be based on the discovery that microparticles containing nucleic acids and having an appropriate size for phagocytosis can be made without adversely affecting nucleic acid integrity. Hedley at col. 1, lines 32-37. Hedley states that phagocytosis of microparticles by macrophages and other antigen presenting cells (APCs) is an effective means for introducing the nucleic acid into these cells. *Id.* at col. 8, lines 13-15. Hedley teaches various means of *in vivo* delivery. *Id.* at col. 8, lines 20-34.

The microparticle-based technique of Hedley, therefore, has certain things in common with the teachings of Song. For example, Hedley describes non-viral gene delivery, while Song teaches the use of any gene delivery vehicle, whether viral or non-viral. Moreover, Hedley's composition is administered *in vivo*, whereas Song teaches the use of any mode of administration, whether *in vivo* or *ex vivo*.

However, the Examiner's argument that Hedley supplements Song through its teachings regarding the use of microparticles to introduce and express antigens in *dendritic* cells *in vitro* is clearly the result of undue hindsight.

In particular, Hedley does speculate at col. 8, lines 25-27 that, during intradermal delivery, microparticles could be *introduced to* antigen presenting cells of the skin, including dendritic cells and Langerhans cells (presumably because they are inherently present in the skin). This, however, is the only mention of dendritic cells in all of Hedley. Elsewhere Hedley refers to

"macrophages and other antigen presenting cells (APCs)." See col. 8, lines 13-14. Moreover, this single mention of dendritic cells is in conjunction with an *in vivo* technique.

In addition, Hedley describes microparticles with internal nucleic acids, rather than microparticles having *adsorbed* antigen-encoding polynucleotide as claimed in claim 54. See, for example, Hedley at col. 1, lines 32-37 ("microparticles *containing* nucleic acids"), *Id.* at col. 9, lines 2-4 ("microparticles can be prepared which carry ... DNA ... *within* each microparticle"), *Id.* at col. 13, lines 64-66 ("the protein or peptide encoded by the nucleic acid contained *within* the microparticle"), *Id.* at Table 5 ("[p]hagocytosis of *encapsulated* DNA leads to expression of a luciferase reporter gene construct") and Table 6 ("[e]xpression of *encapsulated* luciferase DNA in murine muscles"). (Emphasis added.)

With respect to the distinction between encapsulation vs. adsorption, the Examiner argues that the claims *encompass* microparticles with encapsulated nucleic acid and points specifically to claims 46 and 50 in which at least a portion of said polynucleotide is entrapped within said microparticles. Office Action mailed September 22, 2004, at page 8. However, this reasoning is clearly erroneous, as the test for obviousness is based on whether or not there is some suggestion or motivation in the prior art to combine reference teachings and arrive at the claimed invention, *not* whether or not the claims might *encompass* certain features of the prior art.

The Examiner further alleges that "the interaction of the polynucleotide with the microparticle depends on the charge characteristics of the microparticle itself and the presence or absence of additional molecules such as detergents or surfactants. The microparticles of Hedley et al. are not positively charged, thus combining the microparticles with the polynucleotide results primarily in encapsulation. On the other hand, Fattal et al. clearly teaches that adding a cationic detergent to the biodegradable microparticles results in particles with a positive charge such that the majority of the negatively charged polynucleotide adsorbs onto the cationic surface rather than encapsulating therein." *Id.* at pages 8-9. With respect to the cationic detergent, the Examiner alleges that Fattal provides motivation for including a cationic detergent in a microparticle by teaching that inclusion of a cationic detergent in microparticles increases the amount of polynucleotide associated with the polymer particles and increase the uptake of the nucleic acid by phagocytosis. *Id.* at page 8.

This line of reason is also erroneous. The polynucleotide of Hedley is encapsulated due to the double (w/o/w) emulsion particle formation process that is performed in Hedley. See col.

14, lines 22-39. The polynucleotide of Hedley is never "combined with" preexisting microparticles. In a completely non-analogous process, Fattal adsorbs a 15-mer antisense oligonucleotide onto nanoparticles by adding cationic detergent and oligonucelotides to a nanoparticle suspension in the presence of NaCl. Fattal at page 138, col. 2.

In this regard, it is respectfully submitted that one of ordinary skill in the art would not have been motivated to draw inferences between the teachings of Song, Hedley and Fattal as urged by the Examiner, because these references each describes a different approach to nucleic acid delivery. For example, Song describes viral techniques as well as non-viral techniques in which polynucleotides are associated with condensing agents or encapsulated in liposomes. Song is silent with respect to microparticles. Hedley describes encapsulation of polynucleotides within microparticles, whereas Fattal teaches adsorption of oligonucleotides onto nanoparticles. With respect to the latter two techniques, at the time of the present invention, DNA adsorption and DNA encapsulation were understood by those of ordinary skill in the art to constitute separate and distinct delivery approaches, with some favoring encapsulation based on the notion that the DNA would be protected from the destructive elements (e.g., nucleases) encountered in the biological milieu, and others favoring adsorption based on the notion that the DNA would be protected from destructive elements (e.g., high shear stresses) encountered in the processing environment. By avoiding an adverse affect on nucleic acid integrity during the encapsulation process (see, e.g., Hedley at col. 14, lines 22-39), both objectives were achieved by Hedley, removing motivation to resort to the teachings of Fattal.

Moreover, Fattal reports the internalization of a 15-mer *oligonucleotide* (oligomer) adsorbed onto nanoparticles, and that the oligomer remains intact for several hours after cell uptake. See, e.g., Fattal Abstract and p. 140, col. 2. Clearly, oligonucleotides *per se* do not function in the same manner as polynucleotides, such as those described in Song and Hedley, which encode and express a polypeptide. Thus, the mere fact that a 15-mer oligonucleotide *remains intact* upon internalization would not have lead to a reasonable expectation that full length nucleic acid vectors such as those described in Song and Hedley would be *expressed*, as this requires, *inter alia*, delivery of a full length polynucleotide to the nucleus.

Indeed, the results shown by Fattal with regard to the 15-mer oligonucleotide are not encouraging in this respect. Specifically, Fattal teaches at page 140 that "[a]bout 20% of the oligonucleotide given *free* ***or*** *delivered by* PIHCA *nanoparticles* were found in the *nuclear*

fraction." (Emphasis added.) Upon reading this, one of ordinary skill in the art would not have been motivated to go to the trouble of adsorbing antigen-expressing polynucleotides to particles, because this effort would *not* have been expected to enhance the delivery of the polynucleotide to the nucleus (which is required for expression to take place in the cell) vis-à-vis the simple administration of a free polynucleotide.

Moreover, the particles of Fattal are *nanoparticles*. See, e.g., Fattal title. The preparation of nanoparticles in Fattal references Courvreur et al (1984), U.S. Patent No. 4,489,055, which describes the methods for making alkyl-cyano-acrylate particles having diameters less than 500 nanometers (0.5 μm). Procedures producing particle sizes of less than 200 nanometers (Example 1), between 300 and 500 nanometers (Example 2), and smaller than 200 nanometers (Example 5) are reported. In contrast, the microparticles of Hedley are much larger, for example, up to 10% may have a diameter of 100 microns (μm) or more. See Abstract. In Example 1 of Hedley, approximately 85% of the microparticles are between 1.1 and 10 microns in diameter. However, as seen in Fig. 2, larger particles are also clearly present in significant quantities.

Due the notable differences between Hedley and Fattal (e.g., adsorption vs. encapsulation, microparticles vs. nanoparticles, 15-mer oligonucleotide vs. antigen-encoding polynucleotide, etc.) and due to the fact that Fattal reports no difference in nuclear-fraction oligonucleotide content between oligonucleotide given free or that delivered by nanoparticles, it is respectfully submitted that Fattal would not motivate one of ordinary skill in the art to include cationic detergent like that described in Fattal in conjunction with a microparticle-based transfection agent like that of Hedley, as alleged by the Examiner, nor would there have been a reasonable expectation of success.

In fact, it is by no means clear, based on the teachings of Fattal, that the a cationic detergent would increase the amount of antigen-expressing polynucleotide associated with microparticles such as those described by Hedley or that it would increase the uptake of the nucleic acid by phagocytosis of such microparticles.

On the other hand, one of ordinary skill in the art would have found various reasons to *avoid* the use of a cationic detergent. For example, detergents are typically added to stabilize emulsions that are commonly used to prepare microparticles and/or to impart desirable physical properties to the finished microparticle powder preparation, for example, the ability to flow freely. Nonionic detergents, in particular, polyvinyl alcohol are commonly used for this purpose

(see, e.g., Example 1 of Hedley). Charged detergents, on the other hand, are less desirable, because they impart undesirable properties such as stickiness to the resulting microparticles. For this reason, one of ordinary skill in the art would have been motivated to avoid the use of cationic detergents such as CTAB. The motivation to avoid cationic detergents would have been reinforced by the fact that nonionic detergents, such as polyvinyl alcohol, are generally known to have reduced toxicity as compared to cationic detergents, such as CTAB.

In this regard, "the Examiner must show reasons that the skilled artisan, confronted with the same problems as the inventor and *with no knowledge of the claimed invention*, would select the elements from the cited prior art references for combination in the manner claimed." *In re Rouffet*, 149 F.3d 1350, 47 U.S.P.Q.2d 1453, 1458 (Fed. Cir. 1998). (Emphasis added.) As seen from the above discussion, this would not occur.

Accordingly, it is respectfully submitted that one of ordinary skill in the art at the time of the invention, upon considering Song, Hedley and Fattal *as a whole*, would not have been motivated to provide a method like that claimed in claim 54, nor would there have been a reasonable expectation or success, absent the hindsight gained from appellant's disclosure.

It is therefore respectfully requested that the rejection of claim 54 in view of Song, Hedley and Fattal be overturned.

Rejection of Claims 1-16, 18-23, 29-31, 33-44, 46, 50, 52 and 53

Claims 1-16, 18-23, 29-31, 33-44, 46, 50, 52 and 53 are also rejected under 35 U.S.C. 103(a) as obvious over Song in view of Hedley and Fattal. This rejection is clearly erroneous for the reasons set forth below.

Claim 1, like claim 54 above, is directed to a method of transfecting dendritic cells. The method comprises incubating dendritic cells and a transfection agent that comprises a polynucleotide (which encodes an antigen associated with a virus, a bacterium, a parasite, a fungus or a tumor) adsorbed on surfaces of microparticles. The incubation is performed *ex vivo* for a time sufficient to transfect the dendritic cells with the polynucleotide, thereby leading to the expression of said antigen. Thus, claim 1 is patentable over Song, Hedley and Fattal for the reasons discussed above with respect to claim 54.

Moreover, unlike claim 54, claim 1 further requires that the transfection agent be formed by a process that comprises: (a) providing microparticles comprising a biodegradable polymer and a cationic detergent, and (b) exposing said microparticles to the polynucleotide.

As noted above, Song describes compositions and methods useful for stimulating an immune response against one or more disease associated antigens by genetically modifying dendritic cells *in vivo* or *ex vivo*. Although Song teaches that the gene delivery vehicle may be viral or non-viral (i.e., that *any* gene delivery vehicle will suffice), and that dendritic cells may be genetically modified *in vivo* or *ex vivo* (i.e., that *any* mode of administration will suffice), there is an unambiguous preference in Song for techniques that are viral and in vivo in nature. Furthermore, the non-viral techniques taught by Song (i.e., polynucleotides associated with condensing agents or encapsulated in liposomes) are different from the delivery vehicle of the present invention (i.e., polynucleotides adsorbed on surfaces of microparticles).

Hedley, on the other hand, is said to be based on the discovery that microparticles containing nucleic acids and having an appropriate size for phagocytosis can be made without adversely affecting nucleic acid integrity. However, dendritic cells are mentioned only once in all of Hedley, and this single mention is in conjunction with an *in vivo* technique. Moreover, Hedley teaches microparticles with internal nucleic acids, rather than microparticles having *adsorbed* antigen-encoding polynucleotide as claimed in claim 1. Moreover, Hedley does not teach or suggest a transfection agent that comprises an antigen-encoding polynucleotide adsorbed on surfaces of microparticles, nor does Hedley teach or suggest a cationic detergent.

Fattal, in contrast to Hedley, does report the adsorption of a nucleic acid onto particles. However, these nucleic acids are 15-mer antisense *oligonucleotides* rather than antigen-expressing polynucleotides, and the particles are much smaller than the particles of Hedley.

As discussed in more detail above, the Examiner has not convincingly shown why one of ordinary skill in the art would have been motivated to disregard Song's clear preference for an *in vivo*, viral technique, and employ an *ex vivo* non-viral method as claimed. Assuming solely for the sake of argument that one of ordinary skill in the art would have been motivated to select a non-viral vector for dendritic cell transfection, the Examiner has not convincingly shown why one of ordinary skill would choose a microparticle-based, non-viral method like that of Hedley over the non-viral methods suggested in Song. Assuming solely for the same of argument that one of ordinary skill in the art would have been motivated to select a microparticle-based non-

viral method, it has not been convincingly shown why one of ordinary skill would choose to adsorb the antigen-encoding polynucleotide based on the teachings of Fattal, rather than entrap the antigen-encoding polynucleotide based on the teachings of Hedley. And assuming solely for the same of argument that one of ordinary skill in the art would have been motivated to select a microparticle-based method in which an antigen-encoding polynucleotide is adsorbed, it has not be convincingly shown why one of ordinary skill would choose to employ a cationic detergent like that described in Fattal to do so.

Moreover, claim 1 is directed to a process that, *inter alia*, comprises incubating the dendritic cells with a transfection agent comprising an antigen-encoding polynucleotide adsorbed on surfaces of microparticles, wherein the transfection agent is formed by a process that comprises: (a) providing microparticles that comprise a biodegradable polymer and a cationic detergent, and (b) exposing said microparticles to a polynucleotide.

As previously noted, Song and Hedley are silent concerning polynucleotide adsorption, and they are also silent regarding cationic detergents. In Fattal, on the other hand, a cationic detergent (CTAB) and a 15-mer oligonucleotide are simply added to nanoparticles in suspension. See, e.g., Fattal, p. 138, col. 2. In other words, this process cannot be said to teach or suggest one in which particles that comprise a biodegradable polymer and a cationic detergent are provided and then exposed to a polynucleotide in order to adsorb the polynucleotide to the particles.

Accordingly, it is respectfully submitted that a *prima facie* case of obviousness has not been established with respect to the presently pending claim 1.

Consequently, it is respectfully requested that the rejection of claim 1, and claims 2-16, 18-23, 29-31, 33-44, 46, 50, 52 and 53 depending therefrom, be overturned.

Rejection of Claims 19-23

Claims 19-23 are rejected under 35 U.S.C. 103(a) as obvious over Song in view of Hedley and Fattal. This rejection is clearly erroneous.

First, claims 19-23 depend from claim 1 and are thus patentable over Song, Hedley and Fattal for the reasons set forth in the prior sections.

Moreover, it is noted that claims 19-23 are directed to a procedure in which dendritic cells are transfected *ex vivo* in accordance with claim 1, and then administered to a vertebrate subject in an amount effective to produce an immune response.

As noted above, Song teaches nothing about microparticles as delivery vehicles and Song expresses a clear preference for direct injection of recombinant retroviruses (see, e.g., Song, page 27, lines 25-27) over the use of *ex vivo* techniques.

With respect to Hedley, the Examiner has referred to column 12 and Example 2 of Hedley as supporting *ex vivo* techniques. At col. 12, lines 23-30, Hedley refers to "in vitro/ex vivo use." However, that use is clearly experimental, as opposed to therapeutic. Hedley states nothing about administering dendritic cells that have been transfected *ex vivo* to a vertebrate subject. Instead, Hedley merely states that "[t]he [mammalian] cells can be either analyzed immediately or recultured for future analysis.") *Id.* Similarly, while Example 2 of Hedley describes an *in vitro* cell study, this is just a prelude to the *in vivo* cell studies in Examples 3 *et seq.* to follow. An *in vitro* cell study with macrophages is far removed from a procedure in which dendritic cells are transfected *ex vivo* in accordance with claim 1, and then administered to a vertebrate subject in an amount effective to produce an immune response, as claimed in claims 19-23.

Indeed, from a therapeutic standpoint, Hedley as a whole is clearly directed to *in vivo* treatment techniques. In this regard, Hedley teaches various methods for *in vivo* delivery including (a) direct delivery into the bloodstream (i.e., by intravenous or intraarterial injection or infusion), (b) subcutaneous injection, (c) intradermal delivery, (d) delivery via the gastrointestinal tract and (e) introduction of microparticles into the lungs. *Id.* at col. 8, lines 20-34. See also col. 13, lines 15-20. All in vitro teachings of Hedley are related to experimental, rather than therapeutic procedures.

Fattal, like Hedley, teaches nothing about administering dendritic cells (or any other cells) that have been transfected *ex vivo* to a vertebrate subject.

Accordingly, it is respectfully submitted that a *prima facie* case of obviousness has not been established with respect to presently pending claims 19-23.

Consequently, it is respectfully requested that the rejection of claims 19-23 be overturned.

CONCLUSION

The references relied on by the Examiner do not support a *prima facie* case of obviousness against any of the appealed claims. Thus, it is respectfully submitted that reversal of the rejections of record is in order.

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Respectfully submitted,

David B. Bonham Reg. No. 34,297

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VIII. CLAIMS APPENDIX

1. (Previously presented) A method of transfecting dendritic cells comprising:

providing dendritic cells;

providing a transfection agent comprising polynucleotide adsorbed on surfaces of microparticles, said transfection agent being formed by a process that comprises: (a) providing microparticles comprising a biodegradable polymer and a cationic detergent, and (b) exposing said microparticles to said polynucleotide, said polynucleotide encoding an antigen associated with a virus, a bacterium, a parasite, a fungus or a tumor; and

incubating the dendritic cells and the transfection agent *ex vivo* for a time sufficient to transfect the dendritic cells with the polynucleotide, thereby leading to the expression of said antigen.

2. (Original) The method of claim 1, wherein the dendritic cells originate from bone marrow.

3. (Original) The method of claim 1, wherein the dendritic cells originate from blood.

4. (Original) The method of claim 1, wherein the dendritic cells originate from a vertebrate subject.

5. (Previously presented) The method of claim 1, wherein the dendritic cells originate from a human subject.

6. (Previously presented) The method of claim 1, wherein the cationic detergent is cetyl trimethyl ammonium bromide.

7. (Previously presented) The method of claim 1, wherein the cationic detergent is cetrimide.

8. (Original) The method of claim 1, wherein the polymer is a poly(α-hydroxy acid).

9. (Original) The method of claim 1, wherein the polymer is a poly(lactide).

10. (Original) The method of claim 1, wherein the polymer is a copolymer of D,L-lactide and glycolide or glycolic acid.

11. (Original) The method of claim 1, wherein the polymer is a poly(D,L-lactide-co-glycolide).

12. (Original) The method of claim 1, wherein the polymer is a copolymer of D,L-lactide and caprolactone.

13. (Original) The method of claim 1, wherein the dendritic cells are cultured for about 5 days prior to transfection.

14. (Previously presented) The method of claim 1, wherein the dendritic cells are cultured for about 5 to about 10 days prior to transfection.

15. (Original) The method of claim 1, wherein the dendritic cells and transfecting agent are incubated for about 24 hours.

16. (Previously presented) The method of claim 1, wherein said polynucleotide is provided in the form of a plasmid.

17. (Cancelled)

18. (Previously presented) The method of claim 1, wherein the antigen is associated with human immunodeficiency virus, herpes simplex virus, hepatitis B virus, hepatitis C virus, human papillomavirus, influenza A virus, meningitis A, meningitis B, or meningitis C.

19. (Previously presented) A method for producing an immune response comprising administering, to a vertebrate subject in need thereof, an effective amount of dendritic cells produced by the method of claim 1.

20. (Original) The method according to claim 19, in which the dendritic cells originate from the vertebrate subject.

21. (Original) The method according to claim 19, in which the dendritic cells originate from a healthy vertebrate subject MHC-matched to the vertebrate subject.

22. (Original) The method according to claim 19, in which the dendritic cells are administered parenterally.

23. (Original) The method according to claim 19, in which the dendritic cells are administered by direct injection into affected tissue.

24-28. (Cancelled)

29. (Previously presented) Antigen presenting dendritic cells made by the method of claim 1.

30. (Previously presented) The method according to claim 1, wherein said microparticles have diameters ranging from about 500 nm to about 30 μm.

31. (Original) The method according to claim 1, wherein said transfection agent contains on the order of 1% w/w polynucleotide.

32. (Cancelled)

33. (Previously presented) The method of claim 1, wherein said polynucleotide encodes a viral antigen.

34. (Previously presented) The method of claim 1, wherein said polynucleotide encodes a tumor antigen.

35. (Previously presented) The method of claim 1, wherein said polynucleotide encodes a bacterial antigen.

36. (Previously presented) The method of claim 1, wherein said polynucleotide encodes a parasitic antigen.

37. (Previously presented) The method of claim 1, wherein said polynucleotide encodes a fungal antigen.

38. (Previously presented) The method of claim 19, wherein said polynucleotide encodes a viral antigen.

39. (Previously presented) The method of claim 19, wherein said polynucleotide encodes a tumor antigen.

40. (Previously presented) The method of claim 19, wherein said polynucleotide encodes a bacterial antigen.

41. (Previously presented) The method of claim 19, wherein said polynucleotide encodes a parasitic antigen.

42. (Previously presented) The method of claim 19, wherein said polynucleotide encodes a fungal antigen.

43. (Previously presented) The method of claim 19, wherein said polynucleotide encodes a human immunodeficiency virus antigen, a herpes simplex virus antigen, a hepatitis B virus antigen, a hepatitis C virus antigen, a human papillomavirus antigen, an influenza A virus antigen, a meningitis A antigen, a meningitis B antigen, or a meningitis C antigen.

44. (Previously presented) The method of claim 19, wherein the detergent is cetyl trimethyl ammonium bromide.

45. (Cancelled)

46. (Previously presented) The method of claim 1, wherein at least a portion of said polynucleotide is entrapped within said microparticles.

47-49. (Cancelled)

50. (Previously presented) The method of claim 19, wherein at least a portion of said polynucleotide is entrapped within said microparticles.

51. (Cancelled)

52. (Previously presented) The method of any one of claims 1-7, 13-23, 29-31, 33-44, 46 and 50, wherein the polymer is a poly(lactide-co-glycolide).

53. (Previously presented) The method of any one of claims 1-15, 19-23, 29-31, 44, 46 and 50, wherein the polynucleotide is an expression vector encoding an antigen associated with a virus, a bacterium, a parasite, a fungus or a tumor.

54. (Previously presented) A method of transfecting dendritic cells comprising: incubating dendritic cells and a transfection agent that comprises a polynucleotide, which encodes an antigen associated with a virus, a bacterium, a parasite, a fungus or a tumor, adsorbed on surfaces of microparticles, said incubating being performed *ex vivo,* for a time sufficient to transfect the dendritic cells with the polynucleotide, thereby leading to the expression of said antigen.

IX. EVIDENCE APPENDIX

None.

X. RELATED PROCEEDINGS APPENDIX

None.